Steady-State and Oscillating Photosynthesis by a Starchless Mutant of *Nicotiana sylvestris*

Kenneth R. Hanson

Department of Biochemistry and Genetics, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504

ABSTRACT

The photosynthetic characteristics of wild type *Nicotiana sylvestris* (Speg. et Comes) were compared with those of a 'starchless' mutant NS458 that contains a defective plastid phosphoglucomutase (EC 2.1.5.1) (KR Hanson, NA McHale [1988] Plant Physiol 88: 838–844). The steady-state rate of net CO_2 assimilation (*A*) was studied as a function of $[CO_2]$, $[O_2]$, irradiance, and temperature. At 30°C with saturating light and $[CO_2]$ and low $[O_2]$, *A* for the mutant was half that for the wild type, whereas in normal air it was 90%. The irradiance and $[CO_2]$ at low $[O_2]$ required for saturation were lower than the values for the wild type. At 2000 microbars CO_2, 30°C, and saturating irradiance *A* for both the mutant and wild type was stimulated on going from 4 to 25% O_2 by at least 13%. Slow oscillations in *A* were readily induced with the mutant but not the wild type, provided irradiance and $[CO_2]$ were saturating and $[O_2]$ was low. The period, which was about 5 minutes at 30°C and decreased by about 0.67 minutes per degree, was an order of magnitude slower than periods reported for other plants at corresponding temperatures. To achieve the full oscillation amplitude both irradiance and $[CO_2]$ had to exceed the minimal levels for steady-state saturation. The slowness and duration of the oscillations and the metabolic simplification introduced by deleting starch synthesis makes the mutant especially suitable for investigating the regulatory processes that generate such oscillations.

The isolation of a mutant of the diploid tobacco species *Nicotiana sylvestris* grossly deficient in plastid phosphoglucomutase activity has been described (4). The kinetic properties of the defective enzyme are such that negligible amounts of starch accumulate during the day in greenhouse grown plants. Unlike the starchless mutants of *Arabidopsis thaliana* lacking in phosphoglucomutase (2) and ADP-Glu pyrophosphorylase (8) activity, the *N. sylvestris* mutant stored sufficient neutral sugars during the day to be able to grow normally under greenhouse conditions (3, 4, 6).

The major reason for isolating the mutant was to provide an experimental subject in which the complex regulatory interactions of photosynthetic metabolism have been simplified: all factors related to the partitioning of fixed C between starch formation and sucrose synthesis have been eliminated (3). In the present paper, the general photosynthetic characteristics of the mutant leaf material are compared with those of wild-type tissue. In the absence of photorespiration and at saturating $[CO_2]$ and irradiance, exceptionally slow and pro-

longed oscillations in CO_2 assimilation are readily induced for the mutant, but not for the wild type.

The study of oscillations in CO_2 assimilation has a long history (10, 17, 28, 29). In 1949 Van der Veen (23, 24) showed that oscillations could be generated by exposing leaves to brief periods of darkness and studied the influence of $[CO_2]$, $[O_2]$, irradiance, and temperature. The appearance and disappearance of an inhibitor of photosynthesis was postulated. Subsequent theories to account for oscillations have been concerned with the competition by the two kinases of the reductive photosynthetic cycle for the same ATP pool (26), delays in ATP regeneration associated with the transthylakoid ΔpH (10, 13), and the feedback control of Pi regeneration in the sucrose synthesis pathway by $Fru-2,6-P_2$ (20, 22). The last explanation implies that oscillations in ATP regeneration follow changes in [Pi] amounting to one-eighteenth or less of the total Pi required by the photosynthetic process (3). Experiments involving Pi feeding, Pi sequestration, and the use of sun and shade conditions have emphasized that oscillations only occur when the Pi supply is limited (5, 18, 19, 27, 28). In a situation where alternative explanation for oscillations are under consideration, the availability of a new experimental subject with distinctive characteristics could prove to be of great value.

MATERIALS AND METHODS

Plant Materials

Plants of wild-type *Nicotiana sylvestris* (Speg. et Comes) and mutant NS 514, isolated as 'starchless' and containing a modified plastid phosphoglucomutase (4, 15), were grown in the greenhouse in pots of a commercial mix of sphagnum moss, perlite, and vermiculite. The plants were watered daily and fertilized weekly with a complete nutrient solution. The data shown for the mutant were obtained with F_3 starchless plants from the first backcross with wild-type (female) of the original M_2 isolate, but additional experiments have been performed with starchless plants from a third backcross. Leaves of similar size were cut from mutant and wild type at about 0830 EDT from plants that had not begun to bolt. The leaves were washed with soapy water and rinsed before punching leaf discs or cutting out a leaf section.

Measurements of Photosynthesis

For steady-state measurements the standard sample was 18 leaf discs (16 mm diameter; total area, 36 cm^2; about 750 mg

fresh weight) punched from three leaves. The discs were at once distributed with their lower sides up in a Petri dish and water was added to wet the discs from below (5 mL). Inverted discs were used because there are twice as many stomata on the under surface of tobacco leaves as on top (9). Photosynthetic rates measured in this way were in the same range as those measured in the ADC chamber (see below). The leaf discs were allowed to adjust to the first conditions for assay for at least 1 h before commencing measurements.

Two types of open system were employed to study steady-state photosynthesis. Where only the gas composition was varied and mutant and wild type were compared directly, a pair of 1 L fan-stirred Plexiglass chambers illuminated from above were used. The flow rate of humidified gas was about 0.5 L min^{-1} through each; relative rates were adjusted to give the same final [CO_2]. Once a steady state was established, four to six duplicate assays (requiring 20-30 min in all) were performed by noting the flow rates and sampling the entering and leaving gas streams (30). At higher [CO_2] the sample size was reduced from 5 to 1 mL to keep the measurements within the linear response range of the infrared gas analyzer and the number of assays was increased to compensate for an increase in the fractional error. To study O_2 stimulations at high [CO_2] and in experiments where the temperature or the irradiance was varied, a jacketed liquid-cooled and fan-stirred Plexiglas chamber (90 mL) illuminated from below was employed (11, 30). The irradiance was adjusted with fiberglass window screens. CO_2 compensation points were also determined with this chamber. When the leaf discs reached steady-state photosynthesis in the appropriate gas at 30°C and 600 μmol photons m^{-2} s^{-1} the ports were closed and after 4 min the chamber gas was sampled. The gas flow was restored for 8 min between determinations ($\times 4$).

Oscillations in CO_2 assimilations were followed by using a temperature-controlled aluminum chamber (ADC, Hoddesdon, UK) that held a 10 cm^2 leaf section with its upper side illuminated (12). Gas flowed over both surfaces (about 1.0 L min^{-1}) and water was supplied through the cut edges of the section.

'Normal air' conditions were approximated as 345 μbar CO_2 and 22% O_2. Mean values are listed $\pm$ standard deviations unless otherwise indicated.

RESULTS

Steady-State Photosynthesis

In normal air at 30°C and 500 μmol photons m^{-2} s^{-1}, the average photosynthetic rate for the mutant was 90% of the wild type. For example, in September the mean rates for the mutant and wild type were, respectively, 12.2 $\pm$ 0.5 and 14.0 $\pm$ 0.6 μmol m^{-2} s^{-1} ($n = 10$ and 10; P < 0.005). Higher values were recorded in May, lower in August, but the mutant to wild-type ratio remained the same.

The variations of photosynthesis with [CO_2] at three different [O_2] levels, 30°C, and 500 μmol photons m^{-2} s^{-1} is shown in Figure 1. This irradiance was saturating for the mutant and almost saturating for the wild type. The data shown were collected in two stages. A reference framework was first con-
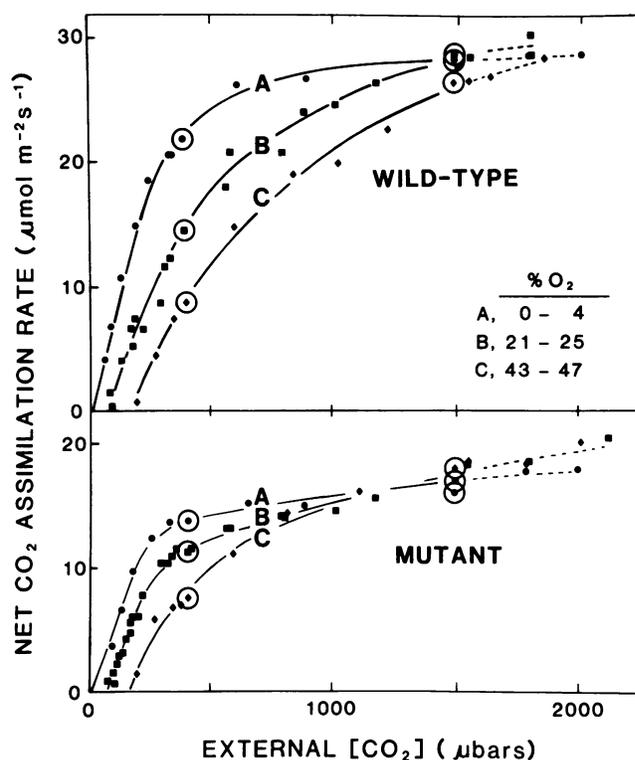
Figure 1. Variations with the external [CO_2] of steady-state net CO_2 assimilation rates for inverted leaf discs of wild-type and mutant plants at low, normal, and high [O_2], 30°C, and 500 μmol photons m^{-2} s^{-1}. The circled reference points were first determined by a series of replicated comparisons ($n = 4$-8). The rate in normal air was first measured and then either the rates in normal [CO_2] and high and low [O_2] or the rates in high [CO_2] and normal, high, and low [O_2]. The means for normal air were calculated and the rest of the data normalized to these means. For normal [CO_2] (345 μbar) and 0.7, 22, and 44% O_2 the normalized rates in μmol CO_2 m^{-2} s^{-1} were MU: 13.7 $\pm$ 1.4, 10.8, and 6.8 $\pm$ 0.4; WT: 20.5 $\pm$ 0.5, 12.0, and 7.4 $\pm$ 0.7. For high [CO_2] (1520 μbar) and 2.7, 24, and 46% O_2 the rates were MU: 16.2 $\pm$ 1.2, 16.9 $\pm$ 1.8, and 18.1 $\pm$ 1.8; WT: 28.6 $\pm$ 2.4, 28.2 $\pm$ 3.7, and 29.7 $\pm$ 6.0. Data collection for the curves proceeded from high to low [CO_2]. Each curve represents data from three experiments. The data from each experiment (about 7 points) were normalized to the 345 μbar CO_2 reference points. The irradiance was fully saturating at high [CO_2] for the mutant and 90% saturating for the wild type.

structed (circled points) by 5 to 8 comparisons between photosynthesis under the conditions indicated and those in normal air. The values for the wild type in high CO_2 overlapped as did those for the mutant. A two-way analyses of variance showed that the high and low O_2 values for the wild type differed significantly (P < 0.05). The means for the mutant in high CO_2 were not significantly different. As each comparison included a normal-air value, the results were normalized to the mean normal-air values. In the second stage, photosynthesis was measured at a series of about seven CO_2 concentrations with [O_2] nominally fixed. For each curve the results from three such sequences were pooled by normalizing the data; hence, the separate sequences are not distinguished by separate symbols in the figure. Sets of four determinations of

CO_2 compensation points were also carried out with a closed system for 11, 22, and 44% O_2 at 30°C and 600 μmol photons m^{-2} s^{-1}. At none of these concentrations were the slight differences between the mutant and wild-type means statistically significant; thus, the results justified extrapolating the curves in Figure 1 to coincide at zero CO_2 for a given O_2 concentration.

At high [CO_2] the assimilation rates were relatively insensitive to changes in [O_2]. This implies that photosynthesis was sink limited (14, 16), primarily by sucrose synthesis in the mutant and by sucrose plus starch synthesis in the wild type (3, 4). Under these conditions, ribulosebisphosphate carboxylase-oxygenase activity is modulated so that the rate of net CO_2 assimilation matches the sink rates (14, 16). The limiting rate for the mutant in low [O_2] was close to 60% of that for the wild type. This ratio decreased to 50% when differences in irradiance response were taken into account. At 345 μbar CO_2 the O_2 inhibition on going from 0.7 to 22% (Fig. 1, A–B) was 41% for the wild type but only 21% for the mutant. The A curves show that at 345 μbar CO_2 and low [O_2] the mutant, but not the wild type, was close to saturation. The transition to saturation for the mutant was much more abrupt than for the wild type. There appeared to be a small increase in photosynthesis with increasing [CO_2] in the region considered to be CO_2 saturated.

Although only a limited number of photosynthetic measurements were made above 1500 μbar CO_2, the general shape of the curves suggested that at 2000 μbar CO_2 there was a small stimulation of CO_2 assimilation on going from 4 to 25% O_2 for both the wild type and the mutant (curves A and B). The explanation for such effects has been the subject of recent debate (7, 17). The matter was, therefore, further investigated by a series of simple steady-state comparisons in which steady-state photosynthesis was measured alternately in 4 and 25% O_2 at 2000 μbar CO_2 and with saturating irradiance at 30°C. Table I shows the O_2 stimulation calculated from sets of three observations beginning with either 4 or 25% O_2. The stimulations were probably greater for the mutant than for the wild type. They were much larger than the small increases observed when photosynthesis was similarly compared at 1500 and 2000 μbar CO_2 at different O_2 concentrations (data not shown). Continuing the alternating sequences for two further sets of steady-state measurements (2 h) showed a decrease in stimulation with time (data not shown). Similar comparisons between photosynthesis at 25 and 47% O_2 showed a drop in photosynthesis for the wild type and a small or negligible increase for the mutant (data not shown).

The variation of photosynthesis with irradiance at 2.7% O_2, saturating [CO_2] (1520 μbar), and at 30°C was investigated (data not shown). The mutant and wild type reached saturation at about 500 and 800 μmol photons m^{-2} s^{-1}, respectively. At the higher irradiance the photosynthetic rate for the mutant was slightly reduced and at low irradiances the quantum efficiency of the mutant was less than for the wild type. No such difference was observed for the *A. thaliana* mutant lacking phosphoglucomutase activity (18). These properties, which have been further investigated using fluorescence-

quenching measurements, will be discussed elsewhere (RB Peterson, KR Hanson, unpublished data).

Figure 2 shows the variation of photosynthesis with temperature at 1.2% O_2, 608 μbar CO_2, and 850 μmol photons m^{-2} s^{-1} and also the variation in normal air at the same irradiance. For the mutant in low [O_2] the conditions were saturating for CO_2 and irradiance at all temperatures and the curve indicates the temperature dependence of limitation by sucrose synthesis. The CO_2 assimilation rate at 30°C was 3.5 times that at 15°C which is comparable to the factor of 2.2 reported for O_2 evolution by barley at saturating CO_2 and irradiance (21). (Barley is also essentially starchless [25]). The low Arrhenius activation energy of about 9.6 kcal mol^{-1} implies that the rate is not set by a single saturated enzyme. As expected, the curves for both mutant and wild type in normal air pass through a maximum, *i.e.* the increase in photorespiration with temperature exceeds the increase in gross CO_2 fixation (11).

Table I. *Stimulations of Photosynthesis on Increasing the O_2 Concentration from 4 to 25% O_2 at 2 mbar CO_2, Saturating Irradiance, and 30°C*

Experiments were performed on sets of 18 leaf discs in the temperature controlled chamber (see "Materials and Methods"). Chamber CO_2 partial pressures were within 40 μbar of 2,000 μbar. The irradiancies were 700 and 1000 μmol photons m^{-2} s^{-1} for the mutant and wild-type experiments, respectively. Different conditions were adopted because of differences in irradiance response (see text). Assays were commenced after a 1 h equilibration period and 8 sets collected (about 40 min). The O_2 concentration was readjusted and the process repeated, and then repeated again after returning to the original [O_2]; total time 4 h. The three sets of observations were compared by a one-way analysis of variance. Different letters appended to the means listed imply significant differences at the P = 0.01 level (Duncan's multiple range test). The standard deviations for the first three results were ±1.1, ±0.9, and ±1.2; others were comparable. Because photosynthesis by the leaf discs changed with time the O_2 stimulation was calculated as the difference between the middle determination and the means of the other two.

[O_2]	Mean Photosynthesis	Mean of First and Third	O_2 Stimulation
%		μmol CO_2 m^{-2} s^{-1}	
Mutant			
Experiment 1			
4	14.7 a		
25	19.7 b	15.4	4.3 (28%)
4	16.1 a		
Experiment 2			
25	15.1 b		
4	12.4 a	14.9	2.6 (21%)
25	14.8 b		
Wild-type			
Experiment 1			
4	32.9 b		
25	36.9 c	31.5	5.4 (17%)
4	30.1 a		
Experiment 2			
25	37.9 b		
4	32.9 a	37.3	4.4 (13%)
25	36.7 b		
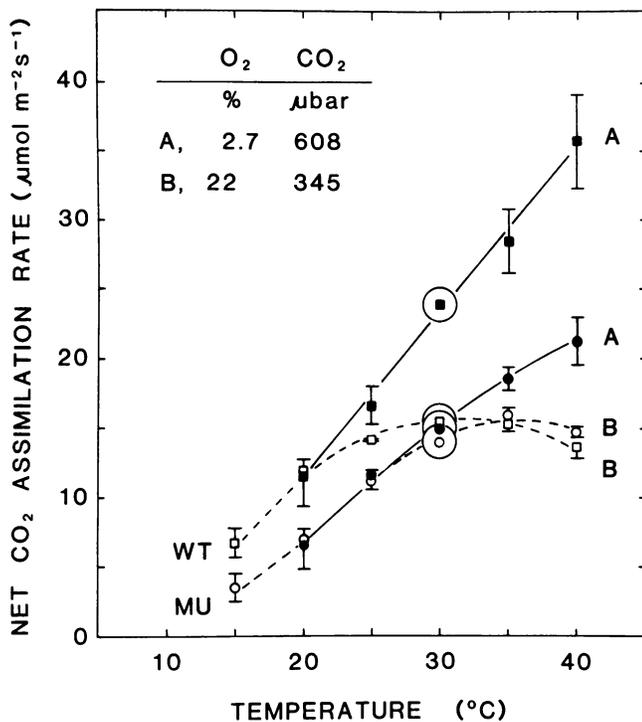
Figure 2. Variation with leaf temperature of steady state net CO_2 assimilation rates at 850 μmol photons m^{-2} s^{-1} for inverted leaf discs of mutant (MU) and wild-type (WT) plants: A, low [O_2] and moderate [CO_2]; B, normal air. The relationship between the 30°C reference points (circled) was established by replicate comparisons as for Figure 1: the rates for conditions A and B were MU: 15.0 and 14.0; WT: 24.0 and 15.5 μmol CO_2 m^{-2} s^{-1}. In collecting data for the curves the initial equilibration was at 30°C. The change on going from 30 to 40°C was reversible. The data were collected in a descending temperature sequence. The data for each sequence were normalized to the value for the appropriate circled reference point. The standard deviations shown are for the normalized data ($n = 3$).

Oscillations

Slow oscillations in CO_2 assimilation by the mutant were readily induced provided both the irradiance and [CO_2] were high and [O_2] was low. Similar results were obtained with inverted leaf discs and with leaf sections in the ADC chamber. The results shown here were obtained with the ADC chamber. It was observed early in this study (4) that the oscillation period at 30°C was about 5 min. As 30°C was within the range of normal greenhouse temperatures, and the periods were inconveniently long at lower temperatures, 30°C was employed for both steady state and oscillation studies. Figure 3 shows that the oscillations by the mutant resemble those reported for other species (19, 28) except for their duration and the length of the period. The 40 min of oscillations in panel 1 were initiated by restoring the CO_2 concentration after a period in the light with no gas flow. The initial peak at 15 s is attributable to the prior accumulation of ribulose bisphosphate. The brief spike before the peak is an artifact of the transit time between reference and measuring cells. Panels 2 and 3 show fluctuations initiated by restoring the light after

a dark period without disrupting the gas flow. The initial peak at 1 min, attributable to compounds awaiting reduction or phosphorylation, was followed by a 4 min flat trough. The highly reproducible sudden rise at about 6 min may indicate a major discontinuity between the initial adjustments and the oscillation mode. Panels 4 and 5 show initiation by step increases in irradiance. The drift indicated by dashed lines in panels 1 and 2 was not observed when the irradiance was 735 rather than 550 μmol photons m^{-2} s^{-1}.

Figure 4 shows the dependence of the oscillatory period upon temperature. The period was not influenced by the method of initiation and at a given temperature the sample variation in the period at a given temperature was not obviously correlated with sample variation in steady-state assim-

TIME (min)
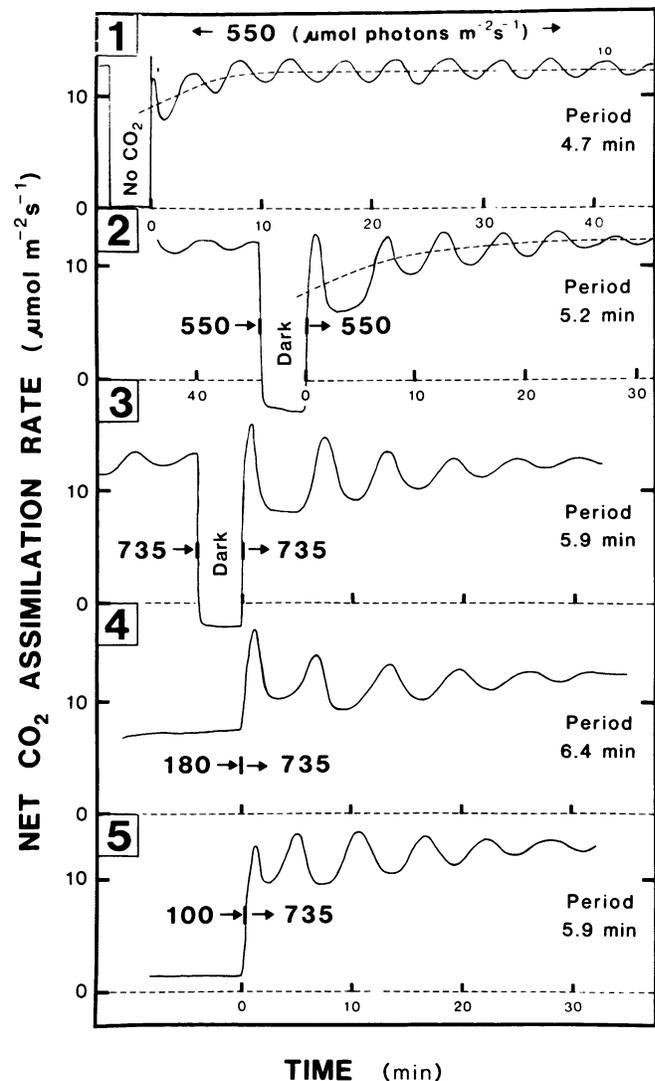
Figure 3. Initiation of oscillations in the net CO_2 assimilation rates for leaf segments of mutant leaves at 30°C in low [O_2] (0.8%) and moderate [CO_2]. For panels 1 and 2, which follow sequentially, the chamber and calculated internal steady state CO_2 concentrations were 347 and 174 μbar, respectively, and for panels 3 to 5 about 404 and 270 μbar.
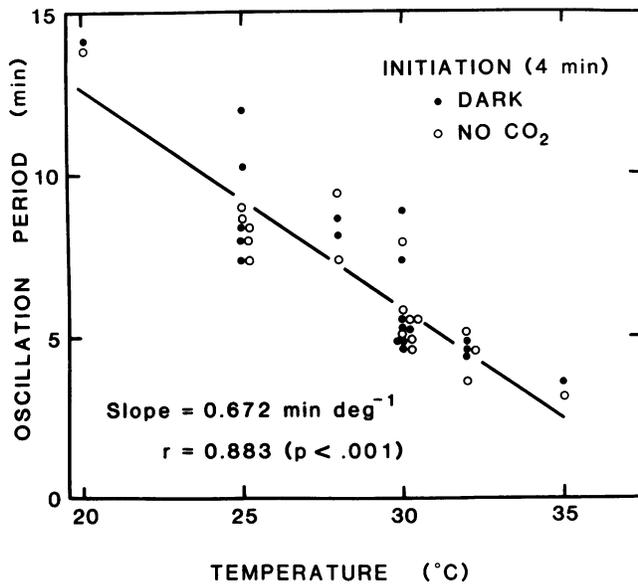
Figure 4. Variation with leaf temperature of the oscillation period under near optimum conditions.

ilation rates. Below 20°C it was difficult to differentiate between drift and oscillatory changes. Because the period at a given temperature was very much longer than the periods reported for other species (see "Discussion"), it was considered possible that at a suitable temperature short-period oscillations would also be observed. There were no signs of such oscillations at 20 or 25°C. The wild type failed to show long- or short-period oscillations at temperatures from 20 to 30°C when tested under a variety of low [O_2], high [CO_2], and high irradiance conditions, including those that readily produce oscillations in spinach (13). (A similar difficulty in obtaining oscillations with soybean has been reported and attributed to the predominance of starch synthesis in this plant [19]). It is, thus, not yet known whether the long period is intrinsic to *N. sylvestris* or whether the period has been changed by the mutation.

Many investigations have shown that light intensity and [CO_2] must exceed threshold values in order to obtain oscillations (19, 24, 28), but the reproducibility of the present system allowed the phenomenon to be studied in greater detail. Figures 5 and 6 show that when the saturation steady-state rates for irradiance (about 450 μmol photons m^{-2} s^{-1}) and [CO_2] (about 230 μbar) were just reached, the oscillation amplitudes were only half the maximum. Maximum amplitudes were reached about 100 μmol photons m^{-2} s^{-1} higher than light saturation was reached and 60 μbar CO_2 higher than CO_2 saturation was reached.

Both of the above results can be interpreted on the assumptions (a) that when irradiance and CO_2 both saturate the rate of photosynthesis depends on the rate of Pi regeneration by the pathway of sucrose synthesis, *i.e.* by sink limitation (14, 16), and (b) that under oscillatory conditions the rate of Pi regeneration oscillates (20, 22). When under steady-state conditions the irradiance is reduced to the minimal amount needed for saturation, the light energy just suffices to regen-

erate ATP from Pi at the rate that Pi becomes available. This rate of ATP regeneration determines the rate of ribulose bisphosphate regeneration and hence the rate of CO_2 fixation, the carboxylase-oxygenase being saturated by CO_2. If Pi availability oscillates about this steady-state value, additional light energy is required for ATP regeneration to match the peak in [Pi]. When the [Pi] falls below the peak level the excess light energy is dissipated. Similarly, for the full [Pi] amplitude to be followed when excess irradiance is supplied [CO_2] must exceed the minimal concentration for steady-state saturation. It must be noted that the results do not exclude the alternative hypothesis that the oscillations arise because of a delay in the ATP regeneration process (10, 13). If this is the case, Figure 5 implies that for maximum amplitude the input energy must exceed the average energy required, *i.e.* there is no energy trade-off between peaks and troughs.

Whereas the higher irradiances did not reduce the amplitude, higher [CO_2] did reduce the amplitude and period (data not shown). At 580 μbar CO_2 the amplitude was not very different from that at 180 μbar CO_2 and about 10% of that at
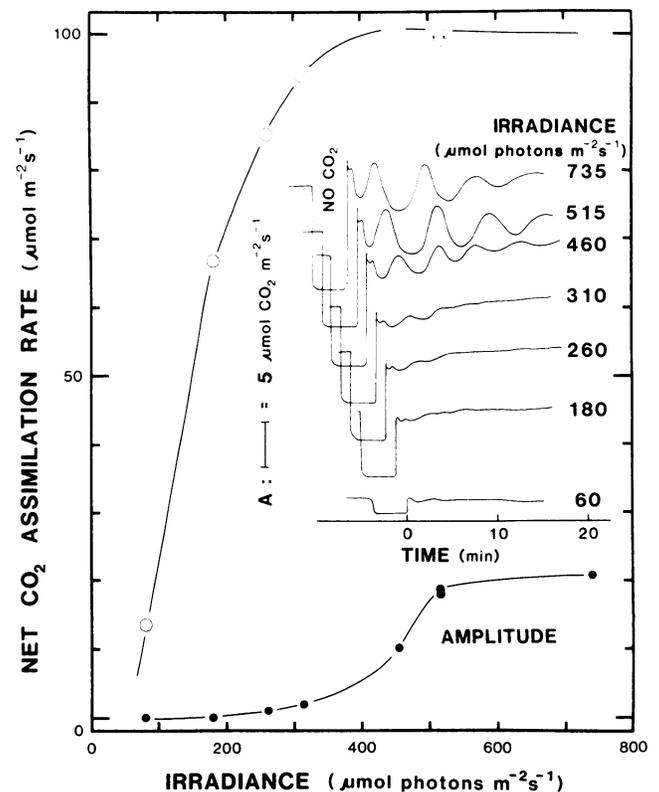
Figure 5. Variation with irradiance of the oscillation amplitudes and steady state net CO_2 assimilation rates for leaf segments of mutant leaves at 30°C in low [O_2] (0.8%) and about 410 μbar CO_2. Rates and amplitudes are expressed relative to the limiting steady-state assimilation rate of 11.6 μmol m^{-2} s^{-1}. Amplitudes were calculated from peaks 2 and 3 only. The oscillation curves were recorded in the order of descending irradiance. The internal [CO_2] was about 260 μbar when the assimilation rate was 100% and increased to 325 μbars as the rate decreased. The periods decreased from 5.5 min at the higher irradiances to 3.0 min at 310 μmol photons m^{-2} s^{-1}.
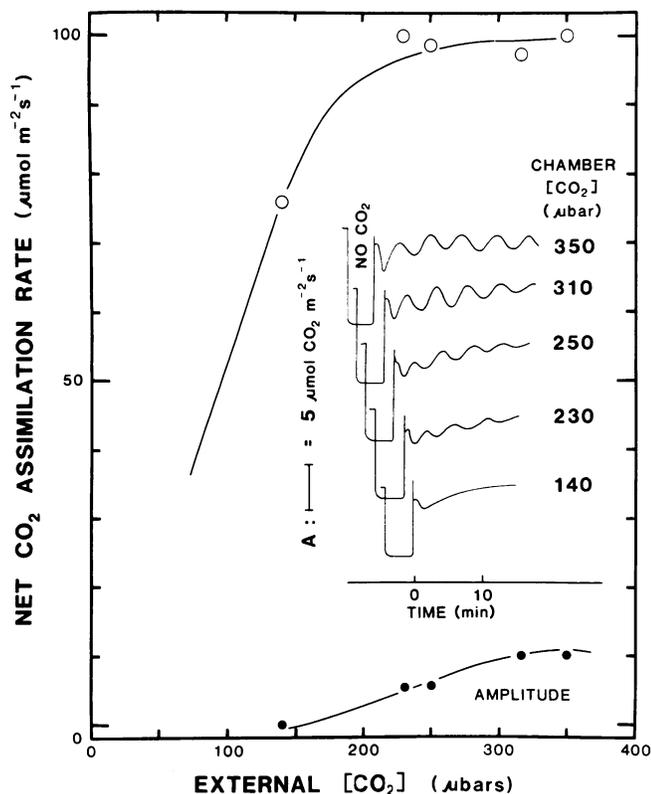
Figure 6. Variation with $[CO_2]$ of the oscillation amplitude and steady state net CO_2 assimilation rates for leaf segments of mutant leaves at 30°C in low $[O_2]$ and 550 μmol photons m^{-2} s^{-1}. Rates and amplitudes are expressed as in Figure 5. The oscillation curves were recorded in the order of decreasing $[CO_2]$. The internal $[CO_2]$ fell from 2180 μbar at the higher two values to 64 μbar at the lowest. The periods decreased from 4.7 to 4.1 min.

385 μbar. This phenomenon was reversible: the amplitude increased on returning to 385 μbar from 580 μbar CO_2. Similar results were obtained when a dark period was used to induce oscillations. The optimum $[CO_2]$ for oscillations varied with the sample, but at 30°C was in the range 350 to 450 μbar CO_2.

DISCUSSION

The result shown in the figures were all obtained with F_3 starchless plants from the first backcross of the starchless mutant with the wild type (4). Recently obtained starchless plants from the third backcross have similar characteristics. It is, thus, highly probable that the difference between wild type and mutant arise only from the modification of the plastid phosphoglucomutase gene.

The steady-state differences between mutant and wild type reported here resemble in some respects those reported for the *A. thaliana* starchless mutants (1, 8). The mutants of both species deficient in plastid phosphoglucomutase activity show much lower photosynthetic rates than the wild type when $[O_2]$ is low and CO_2 and irradiance are saturating, but these differences are minimized in normal air (1; Fig. 2). The

mutants of both species in low $[O_2]$ were saturated at lower CO_2 concentrations and irradiances than the wild type (18). Sivak and Rowell have argued that these and other characteristics are associated with a reduced availability of phosphate (18). The simplification introduced by deleting starch synthesis may make it easier to determine how phosphate availability limits the photosynthetic rate and how phosphate is apportioned between metabolism and vacuolar reserves.

The finding that both the mutant and the wild type were stimulated by O_2 at high CO_2 is suprising (Table I). Sharkey and Vassey (17) showed that the analogous O_2 stimulation of photosynthesis by potato leaves at 22.5°C could be explained by an increase in starch synthesis with sucrose synthesis remaining essentially constant. Evidence was presented that starch synthesis increased because increased photorespiration led to a decreased concentration of 3-phosphoglycerate and thus to a reduction in the inhibition of plastid phosphgluco-isomerase. In the *N sylvestris* mutant, therefore, there should have been no stimulation. As the plastid phosphoglucomutase is defective (4), rather than absent, some starch synthesis may occur at high $[CO_2]$, but it seems unlikely that this explains the observed stimulation. If the changes in the stromal metabolites brought about by photorespiration influence the export of triose-P in exchange for Pi, the rate of sucrose synthesis could be increased (7). This assumes that sink limitation by sucrose synthesis involves both the phosphate transporter and the cytosolic pathway of sucrose synthesis and not just the latter. When starch and sucrose synthesis can occur, as in the potato leaves, starch synthesis may be favored.

The *N. sylvestris* mutant is unique in one important respect. The oscillations induced in the mutant (Fig. 4) are, at a given temperature, an order of magnitude slower than those induced in most other plants that have been studied. Typical published values are: barley, 2 min at 15°C, and 1 min at 20°C (22, 29); spinach, about 2.5 min at 11°C (7), 2 min at 20°C (29), and 20 s at 26°C (2); bean (*Phaseolus vulgaris*) 1.25 min at 23°C (14). The bean *Vicia faba* is unusual in having a period of 5 to 6 min at 25°C (10). The period for the *A. thaliana* mutant lacking phosphoglucomutase and also for the wild type was <2 min at 20°C (18). It seems unlikely that the oscillations of the *N. sylvestris* mutant are generated by an entirely independent metabolic mechanism as there were no indications of additional short-period oscillations at 20 or 25°C, the initiation phenomena and damping patterns were similar to those reported for other plants, the requirement that irradiance and $[CO_2]$ should be saturating is common to all the examples studied, and $[O_2]$, as for other plants, reduced the tendency to oscillate (19, 28).

It was noted in the introduction that different explanations have been advanced to account for oscillations in photosynthesis. Oscillation could be generated in the cytosol, the chloroplast, or by both in combination. The task of distinguishing between these alternatives is intrinsically difficult because changes generated in the one compartment must be followed by the other, *e.g.* if the oscillations result from the dependence of ATP regeneration on the transthylakoid ΔpH (10, 13), both fluorescence quenching and the levels of cytosolic compounds will fluctuate; if they result from the feedback regulation of sucrose synthesis by Fru-2,6-P_2 (20, 22),

fluorescence quenching will fluctuate in response. The *N. sylvestris* mutant is important because the time constants for some part of the overall system differ from those for the plants usually studied; hence, the dynamic relationship between cytosol and chloroplast must be different. This topic will be discussed further when the results of fluorescence quenching studies are reported (RB Peterson, KR Hanson, unpublished data). It seems possible that in general the oscillations are determined by both the chloroplast and the cytosol and that the *N. sylvestris* mutant is a limiting case in which the cytosol is dominant. If oscillations in Pi release generated in the cytosolic pathway of sucrose synthesis are slow enough, plastid metabolism could follow Pi release in a quasi-equilibrium manner.

ACKNOWLEDGMENTS

LITERATURE CITED

1. **Caspar T, Huber SC, Somerville C** (1985) Alterations in growth, photosynthesis, and respiration in a starchless mutant of *Arabidopsis thaliana* (L.) deficient in chloroplast phosphoglucomutase activity. Plant Physiol **79:** 11–17
2. **Furbank RT, Foyer CH** (1986) Oscillations in levels of metabolites from the photosynthetic carbon reduction cycle in spinach leaf discs generated by the transition from air to 5% CO_2. Arch Biochem Biophys **246:** 240–244
3. **Hanson KR** (1990) Regulation of starch and sucrose synthesis in tobacco species. Studies of *Nicotiana tabacum* and a mutant of *N. sylvestris* deficient in plastid phosphoglucomutase. *In* I Zelitch, ed, Perspectives in the Biochemical and Genetic Regulation of Photosynthesis. New York, Wiley-Liss, pp 69–84
4. **Hanson KR, McHale NA** (1988) A starchless mutant of *Nicotiana sylvestris* containing a modified plastid phosphoglucomutase. Plant Physiol **88:** 838–844
5. **Harris GC, Cheesbrough JK, Walker DA** (1983) Measurement of CO_2 and H_2O vapor exchange in spinach leaf disks. Plant Physiol **71:** 102–107
6. **Huber SC, Hanson KR** (1989) Carbon partitioning and growth of a starchless mutant of *Nicotiana sylvestris* (abstract No. 1043). Plant Physiol **89:** S-174.
7. **Leegood RC, Furbank RT** (1986). Stimulation of photosynthesis by 2% oxygen at low temperatures is restored by phosphate. Planta **168:** 84–93
8. **Lin T-P, Caspar T, Somerville C, Preiss J** (1988) Isolation and characterization of a starchless mutant of *Arabidopsis thaliana* (L.) Heynh lacking ADPglucose pyrophosphorylase activity. Plant Physiol **86:** 1131–1135
9. **Meidner H, Mansfield TA** (1968) Physiology of Stomata. Maidenhead, UK, McGraw-Hill, p 5
10. **Ogawa T** (1982) Simple oscillations in photosynthesis of higher plants. Biochim Biophys Acta **681:** 103–109
11. **Peterson RB** (1983) Estimation of photorespiration based on the initial rate of postillumination CO_2 release. II. Effects of O_2, CO_2, and temperature. Plant Physiol **73:** 983–988
12. **Peterson RB** (1989) Partitioning of noncyclic photosynthetic
13. electron transport to O_2-dependent dissipative processes as probed by fluorescence and CO_2 exchange. Plant Physiol **90:** 1322–1328
13. **Peterson RB, Sivak MN, Walker DA** (1988) Carbon dioxide-induced oscillations in fluorescence and photosynthesis. Role of thylakoid membrane energization in regulation of photosystem II activity. Plant Physiol **88:** 1125–1130
14. **Sage RF, Sharkey TD, Seemann JR** (1988) The in-vivo response of the ribulose-1,5-bisphosphate carboxylase activation state and the pool sizes of photosynthetic metabolites to elevated CO_2 in *Phaseolus vulgaris* L. Planta **174:** 407–416
15. **Salvucci ME, Drake RR, Broadbent KP, Haley BE, Hanson KR, McHale NA** (1990) Identification of the 64 kilodalton chloroplast stromal phosphoprotein as phosphoglucomutase. Plant Physiol **93:** 105–109
16. **Sharkey TD, Seemann JR, Berry JA** (1986) Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to changing partial pressure of O_2 and light in *Phaseolus vulgaris*. Plant Physiol **81:** 788–791
17. **Sharkey TD, Vassey TL** (1989) Low oxygen inhibition of photosynthesis is caused by inhibition of starch synthesis. Plant Physiol **90:** 385–387
18. **Sivak MN, Rowell J** (1988) Phosphate limits photosynthesis in a starchless mutant of *Arabidopsis thaliana* deficient in chloroplast phosphoglucomutase activity. Plant Physiol Biochem **26:** 493–501
19. **Sivak MN, Walker DA** (1987) Oscillations and other symptoms of limitation of *in vivo* photosynthesis by inadequate phosphate supply to the chloroplast. Plant Physiol Biochem **25:** 635–648
20. **Stitt M** (1987) Limitation of photosynthesis by sucrose synthesis. Prog Photosynth Res **10:** 685–692
21. **Stitt M, Grosse H** (1988) Interactions between sucrose synthesis and CO_2 fixation IV. Temperature-dependent adjustments of the relation between sucrose synthesis and CO_2 fixation. J Plant Physiol **133:** 392–400
22. **Stitt M, Grosse H, Woo K-C** (1988) Interactions between sucrose synthesis and CO_2 fixation II. Alterations of fructose 2,6-bisphosphate during photosynthetic oscillations. J Plant Physiol **133:** 138–143
23. **Van der Veen R** (1949) Induction phenomena in photosynthesis, I. Physiol Plant **2:** 217–234
24. **Van der Veen R** (1949) Induction phenomena in photosynthesis, II. Physiol Plant **2:** 287–296
25. **Wagner W, Wiemken A** (1989) Fructan metabolism in expanded primary leaves of barley (*Hordeum vulgare* L. cv. *Gerbel*): change upon ageing and spatial organization along the leaf blade. J Plant Physiol **134:** 237–242
26. **Walker DA** (1981) Photosynthetic induction. *In* G Akoyunoglou, ed, Photosynthesis, Vol IV. Regulation of Carbon Metabolism (Proceedings of the Fifth International Congress on Photosynthesis). Balban, Philadelphia, pp 189–202
27. **Walker DA, Osmond CB** (1986) Measurement of photosynthesis *in vivo* with a leaf disc electrode: correlations between light dependence of steady-state photosynthetic O_2 evolution and chlorophyll *a* fluorescence transients. Proc Trans R Soc Lond B Biol Sci **227:** 267–280
28. **Walker DA, Sivak MN** (1986) Photosynthesis and phosphate: a cellular affair? TIBS **11:** 176–179
29. **Walker DA, Sivak MN, Prinsley RT, Cheesbrough JK** (1983) Simultaneous measurement of oscillations in oxygen evolution and chlorophyll *a* fluorescence in leaf pieces. Plant Physiol **73:** 542–549
30. **Zelitch I** (1989) Selection and characterization of tobacco plants with novel O_2-resistant photosynthesis. Plant Physiol **90:** 1457–1464